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This report comprises two experimental studies, which are briefly summarized below and are each being prepared for journal publication in the near future.

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I. Effect of Norepinephrine Infusion on Phospholipid Composition of Blood Plasma and Whole Red Blood Cells in Various Part of the Circulation of Anesthetized Dogs by H. P. Schwarz, J. Spitzer and L. Dreisbach.

a. Effect of Norepinephrine on the Phospholipid Composition of Blood Plasma. The results of these experiments are shown in Table I. Table I demonstrates that differences of phospholipid levels in the plasma existed already in different parts of the circulation prior to the infusion of epinephrine. Thus significant increases ($p < 0.01$) of phosphatidylethanolamine, phosphatidylglycerol, phosphoinositides, and sphingomyelins in plasma from the sagittal sinus over that of arterial plasma are demonstrated. There was only a comparable increase of sphingomyelin in the plasma from the hepatic vein over the arterial plasma noted before the norepinephrine application.

The norepinephrine infusion caused a very significant increase (33%) of phosphatidylglycerol and rather slight elevation of phosphatidic acid in the plasma from the aorta. Plasma samples taken after the infusion showed elevation of phosphatidylethanolamine, and sphingomyelin as well as lesser increases of lecithin, phosphoinositides and alkyl ethers across the brain.

b. Effect of Norepinephrine Infusion of the Phospholipid Composition of Whole Red Blood Cells. (Table II). Comparison of Table I and Table II demonstrates that while the lecithin content of the whole red cell is below that of the blood plasma, other phosphatides such as phosphatidylglycerol, phosphatidic acid, phosphatidylethanolamine and phosphatidylserine are three to over ten times greater in the red blood cells. This finding extensively investigated first in this laboratory has important implications, one of them being greater amounts of the small individual phosphatides available for the analyses.

Analyses of the samples from different parts of the circulation showed that phosphatidylglycerol and phosphatidic acid were elevated in the sagittal sinus already prior to the norepinephrine infusion and that a similar elevation of phosphatidylglycerol across the splanchnic area

existed at that time. The norepinephrine infusion caused a very significant elevation of phosphatidylglycerol by almost 40% in the red blood cells from the aorta. There was, however, no change of that phospholipid across the brain and the splanchnic area following the norepinephrine infusion.

and II. Phospholipid Composition of Blood Plasma, Whole Red Cells, and "Ghosts" from Control Volunteers, Persons with Sickle Cell Disease - without Crisis and in Crisis, by H. P. Schwarz, M. Dahlke, and L. Dreisbach. This study was carried out in heparinized blood from healthy adult volunteers, adult patients with sickle cell disease, proven by hemoglobin electrophoresis on cellulose acetate and samples taken during crisis consisting of visceral, bone or muscle pain lasting for more than 48 hours and requiring hospital visits or hospitalization. The size and hemoglobin content of the erythrocytes as determined by the Coulter Model S were within normal range. The preparation of the samples and analytical techniques were described in previous reports. The results of the investigation are summarized in Table III.

a. Changes of the Phospholipid Composition of the Blood Plasma in Sickle Cell Disease with and Without Crisis. Comparison of the phospholipid values of sickle cell cases without crisis with those of healthy controls shows a slight lowering ($p < 0.05\%$) of the total lipid phosphorus and lecithin figures, as well as a more significant increase of the plasmalogens in the patients without crisis. In sickle cell crisis a very significant increase of phosphatidylglycerol in the plasma is demonstrated. The elevation amounted to 27% of the value of the control GPG or 46% of the figure found in the cases without crisis. There was, furthermore, a quite significant increase of phosphatidylserine in the plasma of the crisis patient. Lowering of total phospholipids and lecithin, as well as increase of plasmalogens was of about the same magnitude in crisis or without it.

b. Phospholipid Composition of Whole Red Blood Cells in Sickle Cell Disease Without and With Crisis. Comparison of the phospholipid values of healthy controls with those of sickle cell patients without crisis shows a slight decrease of lecithin and serum plasmalogen of the whole red blood cells. Study of the values from cases in crisis indicate somewhat higher lecithin figures, besides slightly lower phosphatidylethanolamine and phosphoinositide and slight changes of the plasmalogens. The very significant increase of phosphatidylglycerol noted in the plasma of sickle cell crisis patients, however, did not occur in the whole red blood cells from these cases.

c. Phospholipid Composition of "Ghosts" from Healthy Controls, Patients with Sickle Cell Disease Without Crisis and in Crisis. Study of the values of phospholipids in "ghosts" from healthy normals shows that 60 to about 66 per cent of most of the individual phospholipids are contained in the "ghosts". The highest amounts in the membrane ("ghosts"), about 75%, is shown by phosphatidylserine and the lowest - 57% by phosphatidylglycerol. In cases of sickle cell disease without crisis, the amounts of certain phospholipids (phosphatidylethanolamine, cardiolipin, phosphatidylserine, phosphatidic acid) in the "ghosts" increased significantly with phosphatidylserine in the membrane amounting to about 96% of the quantity contained in the whole erythrocytes. As an exception only phosphatidylglycerol, sphingomyelin, and serine plasmalogens were found lower in the "ghosts" of patients not in crisis.

The elevation of the individual phosphatides bound to the membrane similarly was noted in crisis patients. Even phosphatidylglycerol found below the control level in the patients not in crisis increased significantly in the "ghosts" under crisis conditions. Only sphingomyelin remained at a lower level under these conditions.

<u>Papers Published in 1974</u>

1. H. P. Schwarz, L. Dreisbach, and J. Spitzer. The Effect of Hemorrhagic Shock on the Phospholipid Composition of Blood Plasma in Anesthetized Dogs, Proc. Soc. Exper. Biol. Med. <u>145</u>, 57 (1974)

2. B. D. Polis, E. Polis, H. P. Schwarz, and L. Dreisbach. The Effect of Cold on the Composition of Phospholipids of the Blood Plasma of Healthy Athletes, Proc. Soc. Exper. Biol. Med., <u>145</u>, 73 (1974)

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TABLE I

Effect of Norepinephrine* Infusion on Phospholipid Composition of Plasma in Various Parts of the Circulation of Anesthetized Dogs[a]

Norepinephrine	Arterial Before	Arterial After	Hepatic Before	Hepatic After	Sagittal Sinus Before	Sagittal Sinus After
Number of Experiments	8		8		8	
Total Lipid Phosphorus	2200 ± 98	2204 ± 91	2244 ± 94	2251 ± 91	2303 ± 93	2322 ± 94
Lecithin	1598 ± 14	1592 ± 20	1613 ± 18	1624 ± 11	1634 ± 18	1662 ± 16^{be}
Phosphatidylethanolamine	26 ± 1	26 ± 1	28 ± 2	28 ± 1	31 ± 1^{cd}	31 ± 1^{ce}
Phosphatidylserine	12 ± 0.3	12 ± 1	11 ± 1	13 ± 0.4	13 ± 0.4	13 ± 1
Cardiolipin	23 ± 1	24 ± 1	26 ± 1	25 ± 1	25 ± 1	26 ± 1
Phosphatidic Acid	10 ± 1	12 ± 1^{bd}	11 ± 0.4	14 ± 1	11 ± 0.4	15 ± 1^{be}
Phosphatidylglycerol	28 ± 1	42 ± 2^{cd}	29 ± 1	42 ± 2	33 ± 1^{cd}	46 ± 2
Phosphatidylinositide	69 ± 2	67 ± 2	73 ± 3	73 ± 1	77 ± 2^{cd}	76 ± 1^{be}
Inorganic Phosphorus*	10 ± 1	11 ± 1	12 ± 1^{bd}	12 ± 1	12 ± 0.5^{bd}	12 ± 1
X-2	9 ± 1	11 ± 1	11 ± 1	12 ± 1	11 ± 1	11 ± 1
Alkali Labile Unknowns	6 ± 1	7 ± 0.4	8 ± 1	9 ± 1	9 ± 1^{bd}	8 ± 1
Choline Plasmalogen	35 ± 1	35 ± 1	37 ± 1	38 ± 2	38 ± 2	37 ± 1
Ethanolamine Plasmalogen	18 ± 2	19 ± 1	19 ± 1	19 ± 2	20 ± 1	20 ± 2
Serine Plasmalogen	10 ± 1	12 ± 1	11 ± 1	11 ± 0.4	12 ± 1	12 ± 1
Unknown Plasmalogen	5 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	7 ± 1
Sphingomyelin	160 ± 2	166 ± 2	173 ± 3^{cd}	175 ± 4	186 ± 3^{cd}	184 ± 3^{ce}
Acid Labile Unknowns	7 ± 1	8 ± 1	10 ± 1	8 ± 1	11 ± 1	10 ± 1
Alkyl Ethers	34 ± 1	35 ± 1	35 ± 1	35 ± 1	37 ± 1	39 ± 1^{be}

*Infused for 60 minutes with 0.4 μg Norepinephrine/kg dog weight/minute

[a]Values are expressed as micromoles per liter, mean ± SEM

[b]p 0.05

[c]p 0.01

[d]Comparison with arterial values before norepinephrine infusion.

[e]Comparison with arterial values after norepinephrine infusion.

TABLE II

Effect of Norepinephrine* Infusion on Phospholipid Composition of Whole Red Blood Cells in Various Parts of the Circulation of Anesthetized Dogs[a]

Norepinephrine	Arterial Before	Arterial After	Hepatic Before	Hepatic After	Sagittal Sinus Before	Sagittal Sinus After
Number of Experiments	8		8		8	
Total Lipid Phosphorus	3152 ± 28	3199 ± 29	3307 ± 49[be]	3345 ± 48[bf]	3321 ± 53[be]	3373 ± 59[bf]
Lecithin	1436 ± 18	1453 ± 25	1526 ± 35	1535 ± 35	1589 ± 45[ce]	1588 ± 49[bf]
Phosphatidylethanolamine	272 ± 6	270 ± 6	278 ± 5	279 ± 5	285 ± 4	288 ± 6
Phosphatidylserine	168 ± 1	167 ± 3	171 ± 4	1172 ± 5	169 ± 5	171 ± 6
Cardiolipin	65 ± 1	66 ± 1	67 ± 1	69 ± 1	69 ± 2	70 ± 2
Phosphatidic Acid	150 ± 2	157 ± 3	156 ± 4	168 ± 4	163 ± 3[ce]	169 ± 5
Phosphatidylglycerol	90 ± 6	135 ± 5[de]	110 ± 3[ce]	143 ± 3	114 ± 3[de]	151 ± 6
Phosphatidylinositide	169 ± 5	173 ± 7	177 ± 8	178 ± 10	182 ± 8	183 ± 8
Inorganic Phosphorus	21 ± 1	20 ± 1	21 ± 1	20 ± 1	20 ± 1	21 ± 1
X-2	18 ± 1	18 ± 2	20 ± 2	17 ± 1	18 ± 1	18 ± 1
Alkali Labile Unknowns	15 ± 1	12 ± 1	13 ± 2	13 ± 2	14 ± 1	12 ± 1
Choline Plasmalogen	124 ± 2	121 ± 5	127 ± 5	130 ± 8	125 ± 4	125 ± 5
Ethanolamine Plasmalogen	70 ± 2	72 ± 2	74 ± 2	73 ± 2	75 ± 2	74 ± 2
Serine Plasmalogen	26 ± 2	30 ± 2	31 ± 3	30 ± 2	31 ± 1[be]	32 ± 1
Unknown Plasmalogen	13 ± 2	14 ± 1	12 ± 1	11 ± 1	12 ± 1	12 ± 1
Sphingomyelin	286 ± 5	301 ± 7	295 ± 6	300 ± 7	306 ± 7	307 ± 8
Acid Labile Unknowns	13 ± 1	15 ± 1	14 ± 1	14 ± 1	15 ± 2	14 ± 1
Alkyl Ethers	73 ± 1	76 ± 2	74 ± 1	73 ± 1	77 ± 1[be]	76 ± 1

*Infused for 60 minutes with 0.4 μg Norepinephrine/kg dog weight/minute
[a]Values are expressed as micromoles per liter, mean ± SEM
[b]p .05
[c]p .02
[d]p .01
[e]Comparison with arterial values before norepinephrine infusion.
[f]Comparison with arterial values after norepinephrine infusion.

TABLE III

Phospholipid Composition of Plasma, Whole Red Blood Cells and "Ghosts" from Control Volunteers and Persons with Sickle Cell Disease - Without Crisis and in Crisis

	PLASMA			CELLS			"GHOSTS"		
	Control	Sickle Cell Patients Without Crisis	Sickle Cell Patients In Crisis	Control	Sickel Cell Patients Without Crisis	Sickel Cell Patients In Crisis	Control	Sickle Cell Patients Without Crisis	Sickle Cell Patients In Crisis
Number of Cases	7	5	7	7	5	7	7	5	7
Total Lipid Phosphorus	2062±59	1828±59[ae]	1827±40[ad]	3906±41	3942±158	4030±66	2540±63	2547±70	2653±43
ecithin	1432±68	1231±36[ae]	1187±23[ad]	1472±25	1335± 37[ac]	1597±58[bc]	951±38	983±54	999±24
Phosphatidylethanolamine	25± 1	27± 3	29± 2	351±12	388± 11	340± 3[bc]	225± 5	280± 3[ac]	261± 6[ac,be]
Phosphatidylserine	11± 1	11± 1	17± 2[be,ad]	182± 5	198± 5	185± 4	138± 4	191± 6[ac]	183± 4[ac]
Cardiolipin	21± 1	20± 1	22± 1	71± 3	80± 2	78± 2	62± 1	75± 6[ae]	74± 2[ac]
Phosphatidic Acid	9±0.3	8± 2	12± 1	156± 3	167± 5	149± 5	95± 5	143± 3[ac]	133± 3[ac,be]
Phosphatidylglycerol	30± 1	26± 2	38± 1[bc,ac]	103± 2	95± 6	114± 7	59± 2	51± 2[ae]	83± 5[ac,bc]
Phosphatidylinositide	61± 2	65± 5	66± 3	175±11	202± 7	154± 9[bc]	110± 5	120±11	110± 2
Inorganic Phosphorus	9± 1	8± 1	11± 1[be]	18± 2	23± 3	22± 3	14± 2	12± 1	14± 1
X-2	9±0.4	6± 1[ac]	10± 1[bd]	14± 2	19± 5	21± 2	14± 1	12± 3	13± 1
Alkali Labile Unknowns	8± 1	4± 1[ad]	7± 1	10± 1	19± 2[ac]	14± 1[ad]	10± 1	7± 1	8± 1
Choline Plasmalogen	37± 3	32± 1	33± 1	172± 5	137± 2[ac]	155± 3[ac,bc]	123± 7	101± 4[ae]	124± 5
Ethanolamine Plasmalogen	23± 1	15± 1[ac]	16± 1[ac]	166±14	182± 14	212±14[ae]	100± 4	93± 7	133± 7[ac,bc]
Serine Plasmalogen	13± 1	10± 1[ae]	10± 1[ae]	56± 3	32± 7[ac]	62± 6[bd]	39± 2	19± 2[ac]	33± 3[bc]
Unknown Plasmalogen	8± 1	4± 1[ad]	5± 1[ae]	21± 2	14± 3	19± 5	11± 1	8± 1	8± 2
Sphingomyelin	298± 9	314± 8	289± 4[bd]	679±14	662± 18	649±19	411±11	340±15[ac]	360±13[ad]
Acid Labile Unknowns	13± 1	9± 2	11± 1	30± 3	25± 5	27± 5	19± 3	11± 2[ae]	11± 2[ae]
'lkyl Ethers	32± 2	34± 1	37± 1	149± 5	156± 3	147± 6	90± 7	58± 6[ad]	63± 2[ac]

[a] Control versus sickle cell
[b] Sickle cell no crisis versus sickle cell in crisis
[c] $p < 0.01\%$
[d] $p < 0.02\%$
[e] $p < 0.05\%$